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AMPHIDINOLIDE-A, A NOVEL ANTINEOPLASTIC MACROLIDE FROM THE MARINE DINOFLAGELLATE AMPHIDINIUM SP.

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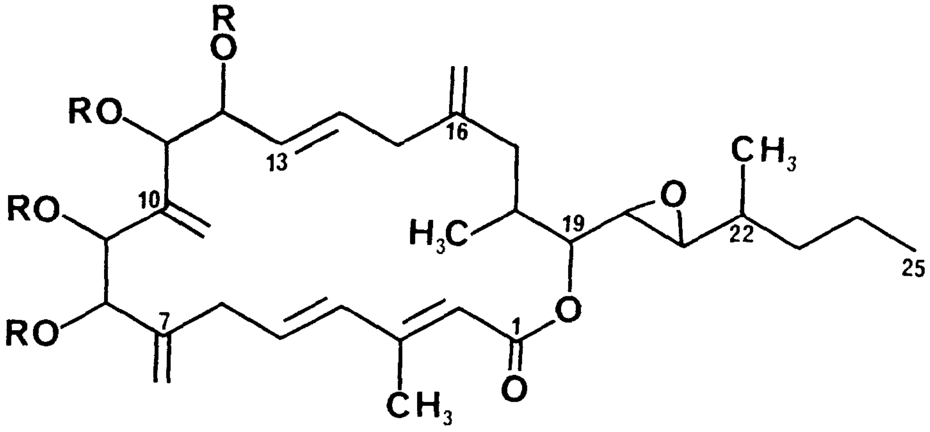
Summary: A novel 20-membered macrolide, amphidinolide-A with antineoplastic activity has been isolated from the marine dinoflagellate Amphidinium sp. and its structure was determined to be 1 by spectral data.

Marine dinoflagellates have been demonstrated to produce chemically interesting and biologically significant metabolites, such as saxitoxin,1) brevetoxin,2) and okadaic acid.3) During our studies on bioactive substances from marine organisms,4) we have investigated extracts of some marine symbiotic microorganisms. In this communication, we describe the isolation and structure assignment of amphidinolide-A (1), a novel antineoplastic macrolide from a laboratory-cultured marine dinoflagellate Amphidinium sp.

The dinoflagellate was isolated from the Okinawan flatworm Amphiscolops sp. and grown unialgally in a sea water medium enriched with ES supplement5) at 25 °C for two weeks. The cultured cells were harvested by centrifugation to yield 375 g of cells from ca. 1000 L of culture. The extracts of harvested cells with methanol-toluene (3:1) were partitioned between toluene and water. The toluene soluble fraction was subjected to repeated silica-gel column chromatographies [methanol/chloroform (5:95) and hexane/acetone (2:1)] followed by reversed phase HPLC (ODS, 88% methanol) to give amphidinolide-A (1, 0.002% yield), which exhibited antitumor activity agaist L1210 (IC_{50} 2.4 μg/mL) and L5178Y (IC_{50} 3.9 μg/mL) murine leukemia cells in vitro, respectively.

Amphidinolide-A (1),6) colorless needles, mp 130-133 °C, $[\alpha]_D^{24}$ +46° (c 1, $CHCl_3$), was shown to have a molecular formula, $C_{31}H_{46}O_7$, by high-resolution EI



1 R = H

2 R = Ac

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR Spectra of Amphidinolide-A (1) (CDCl$_3$, δ in ppm)

Position	H	C$^{a,b)}$	Position	H	C$^{a,b)}$
1		165.81 s	13	5.51 dd	130.96 d
2	5.80 s	118.62 d	14	5.69 dt	130.63 d
3		152.75 s	15	2.76 br s (2H)	39.78 t
Me-3	2.27 s	13.93 q	16		147.26 s
4	6.27 d	136.36 d	CH$_2$=16	4.79 s	112.79 t
5	6.09 ddd	134.72 d		4.88 s	
6	3.20 dd	38.96 t	17	2.35 dd	36.26 t
6'	3.13 dd		17'	1.92 dd	
7		144.88 s	18	2.17 m	33.37 d
CH$_2$=7	5.21 s	114.67 t	Me-18	1.06 d	15.81 q
	5.36 s		19	4.72 dd	70.62 d
8	4.42 br s	72.53 d	20	2.85 dd	54.27 d
9	4.58 br s	74.80 d	21	2.76 br s	61.85 d
10		145.05 s	22	1.38 m	35.36 d
CH$_2$=10	5.37 s	116.02 t	Me-22	0.93 d	14.93 q
	5.49 s		23	1.25-1.55 m (4H)	36.69 t
11	4.09 br s	73.51 d	24		19.96 t
12	4.22 br s	75.77 d	25	0.91 t (3H)	14.23 q

J(H,H) in Hz: 4,5=15.6; 5,6=4.8; 5,6'=8.8; 6,6'=14.6; 12,13=4.1; 13,14=15.4; 14,15=7.7; 14,15'=7.7; 17,17'=14.0; 17,18=5.5; 17',18=9.4; 18,Me(18)=7.0; 18,19=3.4; 19,20=5.9; 20,21=1.9; 22,Me(22)=6.0; 24,25(3H)=6.8.

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a) Multiplicities were determined by DEPT data at 22.5 MHz.

b) Assignments were carried out on the basis of selective proton decoupling experiments at 22.5 MHz.

mass spectrum (Found m/z 530.3210, Δ-3.1 mmu). The UV absorption maximum at 265 nm (ϵ 18000, MeOH) indicated the presence of an $\alpha,\beta,\gamma,\delta$-dienoate chromophore. ^{13}C NMR spectrum of **1** revealed the presence of the following groups: 4 x $-CH_3$, 5 x $-CH_2-$, 2 x $-\overset{|}{C}H-$, 7 x $-\overset{|}{C}H-O$, 3 x $=CH_2$, 5 x $=CH-$, 4 x $=\overset{|}{C}-$, and 1 x $-CO_2-$. This accounts for 31 carbons and 42 protons. The four missing protons belong to 4 hydroxyl groups which were confirmed by the acetylation of **1** to the tetraacetate [**2**, m/z 698 (M^+)].[7] Out of 7 methinoxy carbon signals, four belong to hydroxy methine groups, one to a lactone terminal, and two remaining signals to the epoxide-bearing carbons, suggested by ^1H and ^{13}C signals (δ_H 2.85 dd and 2.76 br s; δ_C 61.85 d and 54.27 d). ^{13}C NMR showed that **1** possesses six double bonds: three belong to exo-methylenes, two to a $\alpha,\beta,\gamma,\delta$-dienoate, and one to an isolated disubstituted double bond.

The detailed analyses of the COSY spectrum of **1** allowed the assignment of all protons (Table 1). The signal for H-2 showed two off-diagonal peaks, one due to allylic coupling to the methyl group on C-3 and the other allylic coupling to H-4. The H-4 signal was coupled to the vicinal proton on C-5 and two allylic protons on C-6. This observation revealed β-methyl-α,γ-diunsaturated ester moiety. The high-field shifted methyl carbon[8] (δ13.93 q) on C-3 and the coupling constant of $J_{4,5}$ (15.6 Hz) indicated 2E,4E-configuration.

The connectivities of the protons from H_2 on C-6 to H_3 on C-25 were clearly established by the COSY spectrum. The exo-methylene protons on C-7, C-10, and C-16 showed obvious cross peaks with the allylic protons on the neighboring carbons. The geometry of $\Delta^{13,14}$-double bond was determined to be E by the coupling constant ($J_{13,14}$=15.4 Hz). The signals for hydroxy methine protons (H-8, 9, 11, and 12) were shifted to lower field [δ5.35 (3H, m) and 5.93 (1H, s)] in the ^1H NMR of the tetraacetate (**2**), while the signal for H-19 was observed at nearly the same chemical shift (δ4.72 for **1** and δ 4.68 for **2**). This fact confirmed that the ester oxygen on C-1 is connected to C-19. The H-19 signal was vicinally coupled to the proton on epoxide-bearing carbon (H-20), which in turn was coupled to the other epoxide-methine proton (H-21) by 1.9 Hz, suggesting the epoxide to be trans.[9] Finally, H-21 was found to be coupled to a methine proton (H-22) which was attached to the carbon with a secondary methyl group and a n-propyl group (C23-C25).

Amphidinolide-A (**1**) may be considered as a 20-membered macrolide with an alkyl side chain, bearing a sort of structural resemblance to swinholide A obtained from marine sponge *Theonella swinhoei*[10] or scytophycin B from blue-green alga *Scytonema pseudohofmanni*.[11] The compound **1** contains several interesting structural features. Three exo-methylene groups on the macrolide ring appear unique and four hydroxyl groups are located closely to each other (C8-C12) to form a hydrophilic part in the molecule.

Studies on the stereochemistry of **1** as well as other accompanying substances are under progress.

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6) *1*: IR (KBr) 3400, 1710, 1625, 1610, 1235, and 1160 cm^{-1}; CD (MeOH) 255 nm ($\Delta\varepsilon$ +7.0); MS (EI) *m/z* 530 (M$^+$), 512, and 494.

7) *2*: ^{1}H NMR (CDCl$_3$)δ 0.90 (3H, t, J=7.0 Hz; H$_3$-25), 0.92 (3H, d, J=6.6 Hz; Me-22), 1.05 (3H, d, J=6.8 Hz; Me-18), 1.99, 2.07, 2.10, and 2.24 (each 3H, s; Ac x 4), 2.33 (3H, s; Me-3), 3.14 (2H, m; H$_2$-6), 4.68 (1H, m; H-19), 4.78 and 4.83 (each 1H, s; CH$_2$=16), 5.02 and 5.07 (each 1H, s; CH$_2$=7), 5.20 (1H, m; H-13), 5.35 (3H, m), 5.36 and 5.48 (each 1H, s; CH$_2$=10), 5.65 (1H, m; H-14), 5.84 (1H, s; H-2), 5.93 (1H, s), 6.17 (1H, m; H-5), and 6.58 (1H, d, J=16.2 Hz; H-4); MS (EI) *m/z* 698 (M$^+$), 670, 638, 610, 578, and 307.

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